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Adhesion strength of the MG63 cell line and its applicability to a fluidised bed bioreactor



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Introduction

The aim of the study was to investigate the strength of adhesion of the MG63 cell line using a tailor made convergent flow chamber. The decreasing cross-sectional area for flow, incorporated in the design of the chamber, increases the linear velocity and thus the shear stress applied to cells within the chamber. The chamber can therefore be used examine cell response to the application of different volumetric flow rates and different shearing media. This design was initially described by Fowler *et al*¹, in which they grew cells on glass slides which were inserted into a similar chamber as the one presented here; the flow chamber geometry allows a range of shear stresses to be tested simultaneously. This work contributes to the development of an experimental system and computational fluid dynamics (CFD) model of a fluidised bed bioreactor for the proliferation of MG63s in a porous hydroxyapatite / tricalcium phosphate (HA/TCP) scaffold.

Chamber Design and Shearing Methodology

The flow chamber, Figure 1, was made from polycarbonate. The base of the chamber in this work was the bottom, internal, surface of a standard T75 flask. Figure 2 is taken from a CFD model of the flow chamber. It shows the variation of the shear stress on the bottom surface of the flow chamber along its centreline, when the volumetric flow rate is set to 80 ml/min. It can be seen that as the cross-sectional area for flow decreases the shear stress increases. The response of the cells to the variation in shear stress has been quantified by using cell count.

MG63 cells were seeded at a density of 20,000 cells/cm². The cells were suspended in 10 ml of complete media. Cells were allowed to attach for either 6 or 24 hours in static conditions at 37°C and 5% CO₂ after which shear stress tests were carried out under three conditions:

- Experiment 1 - Figure 3:** Six hours attachment. Phosphate buffered saline (PBS) at 80 ml/min for 10 minutes, then 150 ml/min for 10 minutes.
Experiment 2 - Figure 4: Six hours attachment. PBS for 10 minutes, then 20 minutes and finally 30 minutes, to make up a total of 60 minutes, all at 28 ml/min.
Experiment 3 - Figure 5: Twenty four hours attachment. Complete media for 30 minutes at 560 ml/min.

In each experiment light micrographs were recorded after each step, the number of adhered cells in each micrograph was counted and the position within the chamber recorded. The shear stress of the fluid was calculated at the mid-point of the flow channel.

Figure 1: Top view of flow chamber, showing converging flow region. Dashed red line denotes line upon which shear stress is calculated in Figure 2 and where the light micrographs were recorded.

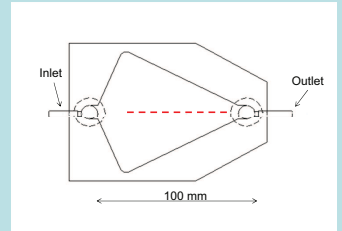
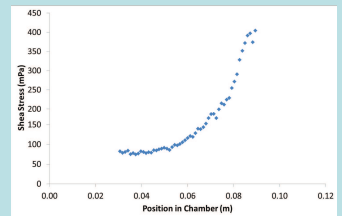


Figure 2: Wall shear stress on the centreline of the flow chamber (dashed line in Figure 1) Volumetric flow rate = 80 ml/min



Results and Discussion

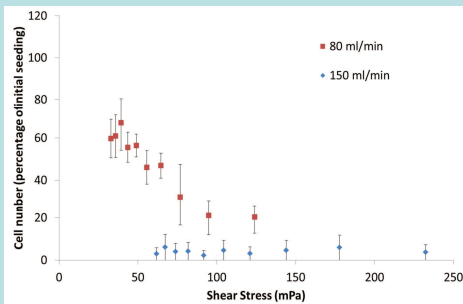


Figure 3: Cell number post PBS shearing at two volumetric flowrates. Error bars are ± 1 s.d., n = 3.

The data shows that the cells are dependent on both the shear stress applied and the time period. The 80 ml/min data shows a decrease in cell number as the shear stress increases, whereas the 150 ml/min data shows a constant, very low, number of cells. The region where the two ranges overlap (25 -125 mPa) would show similar numbers of cells if the detachment was only dependent upon the shear stress, this data shows it is also time dependent.

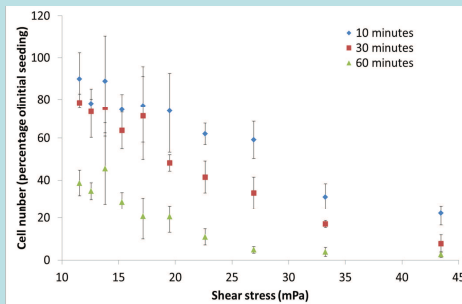


Figure 4: Cell number post PBS shearing over a total of 60 minutes shearing. Error bars are ± 1 s.d., n = 3.

The data shows that for each successive shear at 28 ml/min more cells have been removed. Each set shows a similar pattern of decreasing cell number with shear stress, with the 60 minutes data levelling off at the same minima as the 150 ml/min data in Figure 4. This shows that at these lower shear stresses, PBS is gradually detaching the cells. Cells seeded for 24 hours showed a similar pattern (data not shown).

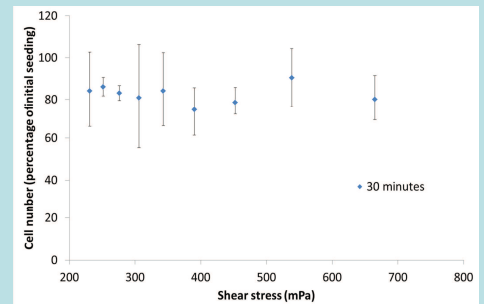


Figure 5: Cell number post media shearing over a total of 30 minutes shearing. Error bars are ± 1 s.d., n = 3.

The data shows that the cells are less easily detached when the shearing media contains foetal calf serum, the shear stresses in this experiment being significantly higher than in the first two experiments. The number of cells is maintaining a relatively constant value, independent of shear stress.

It was observed that many of the cells in this test maintained their filopodia, whereas cells sheared with PBS were observed to 'ball up' as they would when being trypsinised.

Calculation of Adhesion Strength

The adhesion strength can be considered to be equal and opposite to the force required to detach all cells. An approximate value can be calculated using equations 1 and 2 as follows:

$$\text{Equation 1: } \tau_w = \frac{6\mu u}{h_t} \quad \text{Equation 2: } F_t = \frac{110 r_c^3 \tau_w}{a}$$

Where: u = linear velocity, μ = viscosity of shearing fluid, h_t = channel height, τ_w = wall shear stress, r_c = cell radius, a = contact area and F_t = total applied force

PBS: Cells were detached by shear stresses of less than 50 mPa, therefore have a maximum adhesion strength in PBS of 4.8×10^{-9} N

Complete Media: Cells were seen to remain attached at 700 mPa, therefore have an adhesion strength in complete media of at least 7.3×10^{-8} N

Conclusions & Future Work

- The flow chamber has been successfully used to provide a varying shear stress to a uniformly seeded surface.
- The adhered cells sheared with PBS were seen to be detached by shear stresses less than 50 mPa, whereas cells sheared with complete media were seen to remain attached at shear stresses up to 700 mPa.
- The detachment of cells sheared with PBS was shown to be time dependent, whereas the cells sheared with growth media maintained a relatively constant value.
- The highest linear velocity used on the cells was 0.3 ms^{-1} . The particles to be used in the fluidised bed bioreactor have a theoretical minimum fluidisation velocity of 0.05 ms^{-1} , therefore it can be hypothesised that the cells will remain adhered during fluidisation.
- Experiments using media without FCS will be performed to investigate the difference between the detachment behaviour in PBS compared to complete media.
- A second base has been produced which will allow disks of HA/TCP to be contained and therefore the shear stress of detachment on this ceramic material will be tested next.